Electronic Supplementary Information

A ratiometric lysosomal pH probe based on the coumarin-rhodamine FRET system

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Table of Contents

1.	Fig. S1 ¹ H NMR spectrum of probe RC1									
2.	Fig. S2 ¹³ C NMR spectrum of probe RC1									
3.	Fig. S3 Infrared spectrum of probe RC1									
4.	Fig. S4 High resolution mass spectrum of probe RC1	4								
5.	Fig. S5 The ratios of fluorescence intensity of RC1 at pH 4.50 after be	eing								
	incubated for different periods									
6.	TableS1The performancesofRC1andother	pН								
	probes5									
7.	Fig. S6 Fluorescence microscopy images of HeLa cells incubated with H	RC1								
	before and after the treatment of dexamethasone									
8.	Fig. S7 Photostability experiment	8								



Fig. S1 ¹H NMR spectrum of probe RC1.



Fig. S2 ¹³C NMR spectrum of probe RC1.



Fig. S3 Infrared spectrum of probe RC1.



Fig. S4 High resolution mass spectrum of probe RC1.



Fig. S5 The ratios of fluorescence intensity of RC1 (1 μ M) at 477 nm and 582 nm (I₄₇₇/I₅₈₂) at pH 4.50 after being incubated in solution (B-R buffer/EtOH = 7/3, v/v) for 90, 95, 100, 105, 110, 115 min at room temperature. λ ex = 420 nm.

Probes	Probe concentration	Solvent	p <i>K</i> a	Detection range	Response time	Reference
$(El)_2N \xrightarrow{(El)_2} (El)_2 \xrightarrow{(El)_2} (El$	1 μM	EtOH: buffer = 3:7 (v:v)	4.98	4.2-6.0	100 s	This work
$(El)_2N$	10 μM	DMSO : buffer = 0.33:99. 67 (v:v)	5.47	4.5-6.0	Several seconds	8

Table S1. The performances of RC1 and other pH probes

(Et) ₂ N O N(Et) ₂	1 μΜ	DMSO : buffer = 1:99 (v:v)	5.16	4.2-6.0	90 s	16
$(El)_2N \longrightarrow O (V) (V) (El)_2$	10 µM	EtOH: buffer = 1:1 (v:v)	3.21	2.2-4.2	250 min	17
$(E1)_2N \xrightarrow{0} (0) (0) (0) (0) (0) (0) (0) (0) (0) (0)$	5 μΜ	EtOH: buffer = 1:9 (v:v)	4.82	4.5-5.5	200 s	18
	10 μM	EtOH: buffer = 1:1 (v:v)	3.52	3.0-4.0	Less than 1 min	26
	10 μM	EtOH: buffer = 1:1 (v:v)	2.10	0.5-3.5	Less than 1 min	29
$(El)_2N \xrightarrow{0} (Cl)_2 \xrightarrow{0} (Cl$	10 μM	EtOH: buffer = 1:9 (v:v)	5.05	4.7-5.7	About 1 min	30
$(Et)_2N \xrightarrow{O} (V) \xrightarrow{V} (V) $	1 µM	EtOH: buffer = 2:8 (v:v)	4.85	4.2-5.2	100 min	57



Fig. S6 (a) Fluorescence microscopy images of HeLa cells incubated with RC1 (3 μ M) before and after the treatment of dexamethasone (50 μ M). First row: before the treatment of dexamethasone (50 μ M), second row: after the treatment of dexamethasone (50 μ M); (b) fluorescence intensity quantitation was analysed by the Image J. The results were presented as means ± SE with replicates n = 3. *, p < 0.05.

Photostability experiment



Fig. S7 Photostability experiment of **RC1** (3 μ M) in HeLa cells. (a) Fluorescence microscope images of HeLa cells after 0, 2, 4, 8 and 16 min of continuous irradiation. $\lambda ex = 405$ nm. First column: fluorescence images at the blue channel (450-550 nm), second column: fluorescence images at the red channel (550-650 nm), third column: bright field images, fourth column: overlay images of first, second and third column; (b) fluorescence intensity in cells at different periods of time. Fluorescence intensity quantitation was analyzed by the Image J. The results were presented as means ± SE with replicates n = 3.